Please add the following new claims:

37. [New] A method of screening a human subject for an increased risk of developing hereditary lymphedema comprising assaying nucleic acid of a human subject for a mutation that alters the encoded amino acid sequence of at least one VEGFR-3 allele in a manner that correlates with the risk of developing hereditary lymphedema.

B 4

38. [New] The method of claim 37, wherein said mutation reduces signaling of the VEGFR-3 receptor.

I. INTERVIEW SUMMARY AND EXPLANATION OF AMENDMENTS

Applicants thank Examiners Reynolds and Sorbello, and further thank Brian Stanton, for their time and helpful suggestions in discussing the case over the telephone. Claims 1-7, 14, and 20 are amended herein. Attached within Exhibit A are marked-up versions of the claims showing changes made by the current amendment. Also attached as Exhibit B is a clean copy of the claims presently pending in the instant application.

Applicants have amended the preamble and other aspects of claim 1 so that the claim more succinctly recites the invention. These amendments are not intended to narrow the scope of the claim. Also, claim 1 has been amended so that the assaying is for a mutation altering the encoded amino acid sequence of at least one VEGFR-3 allele of a human subject *in a manner that reduces signaling of the VEGFR-3 polypeptide encoded by the allele*. Support for this amendment can be found throughout the specification, including at page 9, line 20, to page 10, line 3. Furthermore, claim 1 has been amended to specify a feature which had been understood by the applicants to be inherent in the original claim, namely, that *absence* of the mutation in the nucleic acid correlates with *no increased risk* of developing hereditary lymphedema. Support for this amendment can be found in the specification at least at page 5, lines 16-29.

During a telephone interview on March 28, which the Applicants acknowledge with thanks, Brian Stanton agreed that the present invention teaches a correlation between mutations in VEGFR-3 and hereditary lymphedema and suggested including a single-step screening claim. The Applicants believe that newly

added Claim 37 (and dependent claim 38) embodies the suggestion that arose from the interview. Support for claims 37 and 38 can be found within the specification at least at page 5, lines 16-26, and page 9, line 20, to page 10, line 3.

Claims 2-6 have been amended also to depend from claim 37. Furthermore, Claims 2-4 have been amended to mirror the more succinct language of amended claim 1.

Claim 7 has been amended to recite "subject" rather than "patient." This amendment is made to provide consistency between the claims. Furthermore, during the telephone interview, the Examiners suggested amending claim 7 to more succinctly recite the steps of a screening method. Applicants have amended claim 7 so that it more succinctly recites the screening method. Support for the amendments can be found within the specification at least at page 9, line 20, to page 10, line 3.

Claims 14 and 20 have been amended to refer to a VEGFR-3 sequence provided in the specification.

The amendments are fully supported by the specification and do not introduce new matter. The Applicants reserve the right to pursue the original claims or their subject matter in related cases, such as continuing applications.

II. RESPONSE TO THE OFFICE ACTION OF OCTOBER 10, 2001

Claims 1-11 and 14-21 were pending and examined in the Office Action dated October 10, 2001. Claims 1-7, 14, 20 have been amended herein and claims 37 and 38 are new. Consequently, claims 1-11, 14-21 and 37-38 are pending and under examination in the instant application and are presented for reconsideration.

In the Office Action, the Examiner allowed claim 19 but maintained the rejection of the remaining claims variously under 35 U.S.C. § 112, first paragraph, for alleged lack of written description and lack of enablement and under 35 U.S.C. § 112, second paragraph, for allegedly failing to particularly point out and distinctly claim the subject matter of the invention. Applicants traverse the rejections.

A. The claims are supported by the written description contained in the specification.

Claims 1-11, 14, 15, 18, 20 and 21 remain rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the

specification in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention. Applicants traverse.

The specification provides a description of the invention which clearly allows persons of ordinary skill in the art to recognize that the instant inventors were in possession of what they claimed. The present invention is directed to, for example, the following method recited in claim 1:

> A method of assaying for risk of developing hereditary lymphedema, comprising assaying nucleic acid of a human subject for a mutation altering the encoded amino acid sequence of at least one VEGFR-3 allele of the human subject in a manner that reduces signaling of the VEGFR-3 polypeptide encoded by the allele; and correlating presence or absence of said mutation in the nucleic acid to a risk of developing hereditary lymphedema, wherein presence of said mutation in the nucleic acid correlates with an increased risk of developing hereditary lymphedema, and wherein absence of said mutation in the nucleic acid correlates with no increased risk of developing hereditary lymphedema.

The present application shows that mutations in VEGFR-3 that interfere with VEGFR-3 signaling have an apparent "causative role in heritable lymphedema" (specification page 39, lines 10-12). The specification provides a human VEGFR-3 coding sequence. (See, e.g., SEQ ID NO:1 and page 8, lines 22.) Given that the present invention teaches that subjects with mutations in VEGFR-3 which diminish receptor function exhibit an increased risk of developing hereditary lymphedema, this reference VEGFR-3 sequence can be used to compare any human subject's VEGFR-3 sequence, as part of the process for looking for mutations that alter the coding sequence for VEGFR-3.

Moreover, the specification provides examples of specific mutations (e.g., see table 3 at page 44) which correlate with hereditary lymphedema. **These are separate mutations in distinct families.** The specification also teaches how to determine the presence or absence of a mutation in VEGFR-3 (see, e.g., page 6, line 16 through to page 8 line 2). Example 1, at pages 28 line 20 through page 29 line 27 of the specification, provides specific protocols of how to determine whether family members are afflicted with hereditary lymphedema and whether a VEGFR-3 mutation correlates with lymphedema development. Thus, the specification has both examples of mutations which are indicative of hereditary lymphedema, as well as specific

instruction as to how one of skill in the art should proceed to identify further such mutations.

These instructions in the specification have been shown to work, as described in Example 5 of the specification. (specification page 43 line 20) "Using procedures essentially as described in Example 1," the Applicants identified "additional mutations . . . correlating strongly with a risk for developing heritable lymphedema" (specification page 44, lines 27-30).

The application also provides biochemical assays to establish whether a particular VEGFR-3 mutation diminishes receptor signaling. For instance, Example 2 in the application describes both ligand-independent and ligand-mediated receptor activation assays to assess the functional effects of newly identified VEGFR-3 mutations.

The Examiner argued that "applicants are in possession of only the VEGFR-3 allele encoded by SEQ ID NO:1 with mutations at nucleotide substitutions at specified positions". As explained above, this analysis is incorrect. The Applicants have identified **several mutations from distinct families** having members that suffer from heritable lymphedema and that showed initial linkage to chromosome 5q. (See Example 5 of the application.)

The Examiner goes on to state that "it is not clear if there is value in screening a population for the presence or absence of mutations which are allegedly reflective of a disease condition when only one allele (out of a whole genus of alleles being present in a population) is known, and described. It is not clear if the other alleles will have other mutations. . . ." As explained in the preceding paragraph, the application itself shows that there is value in screening a population for such mutations. After the P1114L mutation was identified in one family and characterized to show both its predictive value and its putative causative role in disease (see Examples 1-2), the Applicants extended their work to other families and found other predictive mutations. (See Example 5.) The application collectively describes at least 5 separate families in which a different mutation in at least one VEGFR-3 allele was predictive of hereditary lymphedema. Thus, the experiments described in the application itself demonstrate that the invention has immediate value beyond the mutations that the Applicants have identified in their initial work.

The invention presently claimed and rejected is a ***screening assay,*** which involves analysis a human subject's VEGFR-3 nucleic acid to determine if the person

has an informative mutation, i.e., a mutation that has significance in disease, and then drawing a valuable conclusion from the analysis, namely, whether the subject's DNA indicates a correlation to normal versus increased risk of heritable lymphedema. As with any screening method, a proper description involves an explanation of how to perform the analysis and how to interpret the results. Such screening methods do not require description of all possible VEGFR-3 alleles (although several are taught in the application); the description in the application of how to analyze a human subject's DNA and draw the proper conclusions is all that is required. The application describes numerous nucleic acid analysis techniques that are available for practicing the assaying step of the method. (See, *e.g.*, specification p. 6, lines 16-33, outlining several established nucleic acid analysis techniques that are suitable.)

The application also describes and exemplifies how to determine if a mutation correlates with a heritable lymphedema. VEGFR-3 functional considerations are discussed (e.g., tyrosine kinase mutants that are apparently causative of lymphedema), and statistical procedures are taught for determining whether newly discovered mutations segregate with familial disease. Thus, the application describes practice of this aspect of the method both at a population and a biochemical level. Without any foreknowledge of all VEGFR-3 alleles, the present application describes completely and adequately how to practice the screening methods of the invention.

The Examiner maintained the rejection of claims 14, 15, 18, 20 and 21. Applicants traverse this rejection. As amended herein, these claims are directed to oligonucleotide probes for identifying polymorphisms in a human Flt4 receptor tyrosine kinase gene, the oligonucleotide comprising 6-50 nucleotides that have a sequence that is identical or exactly complementary to a portion of a human VEGFR-3 gene sequence set forth in SEQ ID NO:1, except for one sequence difference. Such oligonucleotides are useful for detecting Flt4 polymorphisms, and the application teaches how to correlate such polymorphisms to heritable diseases. Since the oligonucleotide is identical to a portion of the VEGFR-3 sequence set forth in SEQ ID NO:1 except for one difference, claim 14 does not require knowledge of all human (mutant) alleles. Rather, it only requires knowledge of a VEGFR-3 sequence provided in the specification as SEQ ID NO: 1. A similar analysis applies to claim 20. Given that production of oligonucleotide probes is a routine matter to those of skill in the art, and the sequence of VEGFR-3 is provided in the specification as filed, there is no requirement to list each and every possible oligonucleotide.

Additionally, it is unclear why the rejection was raised at all in relation to claims 15 and 21, which define oligonucleotides relative to specific portions of SEQ ID NO: 1 that relate to tyrosine kinase domains. The application establishes that there is especially high relevance to screening for mutations in these domains.

In light of the above comments, the materials and methods of the present invention are fully described in the specification as filed, and as such, the rejection under 35 U.S.C. § 112, first paragraph, should be withdrawn.

B. The claimed invention is enabled by the specification as filed.

The Examiner maintained the rejection of claims 1-11, 14, 15, 18, 20 and 21 under 35 U.S.C. §112, first paragraph, alleging that the application does not enable any person skilled in the art to which it pertains to make and use the invention commensurate in scope with the claims. The Applicants respectfully traverse.

With respect to claims 1-11, the Examiner "argues that the language of the claim recites the following 'presence, **or absence of a mutation altering amino acid sequence**', which embodies the fact the absence of a mutation may confer an altered VEGFR-3, which may in turn confer susceptibility to lymphedema." (Office Action page 6). Applicants have amended the claim 1 such that it specifically recites "wherein absence of said mutation in the nucleic acid correlates with no increased risk of developing hereditary lymphedema." This amendment is not intended as a narrowing amendment, but rather a statement of what was inherent and would have been apparent to a reader of ordinary skill in the art. This amendment obviates the rejection, and the applicants request that it be withdrawn.

As a further basis for rejection, the Examiner also alleged that the application was not enabling "for prediction of any and all familial lymphedemas." This allegation, even if true, does not support a rejection of any claim. The claim does not purport to predict any and all lymphedemas. Rather, the invention purports to predict lymphedemas that can be predicted from VEGFR-3 mutations, which is apparent from a reading of the steps of the claims.

The Examiner doubts that "any 6 nucleotide sequence will appropriately and accurately be predictive of any disease" (Office Action, page 6). Applicants respectfully traverse.

At the outset, the Applicants would urge that the proper analysis is not whether an oligonucleotide can be predictive of a disease, but whether the oligonucleotide can be used for a sequence determination, from which a disease prediction can be made. As early as 1992, those of skill in the art were able to analyze and compare nucleic acid sequences by hybridization to arrays of oligonucleotides. Sequencing by hybridization uses the principles of complementary base-pairing rules and the ability to distinguish a nucleic acid strand that is perfectly complementary from one that is not. For example, by hybridizing one nucleic acid strand of known sequence (sometimes termed a nucleic acid probe) to a second strand of unknown sequence (sometimes termed a sample nucleic acid), one can deduce the sequence of the unknown sample from the known sequence of the nucleic acid probe. Attached herewith as Exhibit C is a copy of Southern et al., (Genomics 13:1006-1017, 1992) which shows that this method provides "a powerful way of comparing related sequences and detecting mutation" (see abstract). While the Southern paper uses octanucleotides, at page 1014 of the same article, the authors describe the Bains and Smith reference in which an "array comprises hexamers". Since the early 1990's others have described use of these techniques in greater detail, such as WO 95/09248 (Exhibit D; see pages 20-29), U.S. Patent No. 6,261,776 (Exhibit E; see claim 2), and U.S. Patent No. 5,744,305 (Exhibit F; see claim 1). In light of these teachings in the art, Applicants submit that 6-mer oligonucleotides are well recognized by those of skill in the art as being useful for determining the presence of a mutation in a give sequence and therefore can be used to determine the presence of a polymorphism in a given gene.

In light of the above comments, Applicants request that the rejection under 35 U.S.C. § 112, first paragraph for lack of enablement be withdrawn and the claims be reconsidered for allowance.

C. The claims are clear and definite and the rejection under 35 U.S.C § 112, second paragraph, should be withdrawn.

The Examiner maintained the rejection of claims 1-11 and 14-17 under 35 U.S.C. § 112, second paragraph, as being allegedly indefinite. Applicants respectfully traverse the rejection.

According to the Examiner "[t]he term "correlates" does not bear the specificity as claimed in the instant invention." Applicants respectfully disagree. One

of skill in the art reviewing the specification would understand the meaning of this term as used in the claims. The term "correlates" is used at page 5, lines 16-26. Moreover, the working examples (especially Example 1) provide the requisite context for "correlates" in discussing the linkage of VEGFR-3 mutations to lymphedema. Applicants have previously provided a Webster's dictionary definition of "correlate" as bearing "...a reciprocal or mutual relationship..." or where two sets or series of things are "...present or set forth so as to show a relation with each other. . .". The patent literature also is replete with the use of this term in a similar context to that being used in the instant application. For example, U.S. Patent No 5,565,323 (Exhibit G) teaches that the presence of at least one mutation in the sequence of a mitochondrial cytochrome oxidase gene **correlates** with the presence or risk of Alzheimer's disease (see claim 1); U.S. Patent No. 5,494,794 (Exhibit H) teaches that a mutation of mitochondrial DNA **correlates** to Alzheimer's disease and/or Parkinson's disease (see claim 1); and U.S. 6,306,576 (Exhibit I) teaches that the presence of elevated levels of a brominated tyrosine species in a test sample **correlates** with the presence of a disease associated with activated eosinophils (see claim 1). Furthermore, scientific literature is replete with the use of this term in the same context. Examples include Grabowski, *Genet Test*, 1(1):5-12, 1997 ("genotype/phenotype **correlations**")(Exhibit J), Schuchman and Miranda, *Genet Test*, 1(1):13-19, 1997 ("genotype/phenotype **correlations**")(Exhibit K), and http://mednews.standford.edu/news_releases_html/1999/junreleases/alztest.html ("mutations are considered highly 'penetrant,' which means they **correlate** strongly with the occurrence of the disease."). The Applicants respectfully submit that in the area of genetic screening involving in which mutations in given genes are found to be linked with or predispose individuals to a given disorder, the term "correlates" is well accepted and conventional nomenclature, and in fact perfectly describes the observations that underlie any genetic test. In this context, the term conveys the nature of the invention with clarity to the reader. In the event that the Examiner maintains the rejection, Applicants request articulation of why "correlate" is considered indefinite, and would welcome discussions with the Examiner regarding alternative acceptable terms that are synonymous with this term.

Claims 14-17 remain rejected for reciting the term "wild type" when referring to the human VEGFR-3 sequence. The claims as amended no longer recite the term "wild type." Therefore, the rejection is moot.

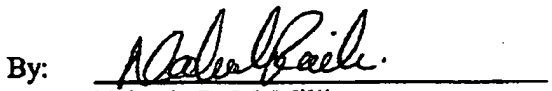
Applicants submit that the claims are clear and the rejection and request that the rejections based on 35 U.S.C. § 112, second paragraph, be withdrawn.

Conclusion

In light of the foregoing amendments and remarks, it is submitted that all claims should be allowed. Should the Examiner wish to discuss any further material of form or substance, she is encouraged to contact the undersigned agent at the telephone number listed below.

Respectfully submitted,

MARSHALL, GERSTEIN & BORUN

By: 

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April 23, 2002

EXHIBIT A

VERSIONS WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

The claims have been amended as follows:

1. [Twice amended] A method of [screening a human subject for an increased] assaying for risk of developing [a] hereditary lymphedema, comprising [the steps of: (a)] assaying nucleic acid of a human subject [to determine a presence or an absence of] for a mutation altering the encoded amino acid sequence [or expression] of at least one VEGFR-3 allele of the human subject in a manner that reduces signaling of the VEGFR-3 polypeptide encoded by the allele; and [screening for an increased risk of developing hereditary lymphedema from the] correlating presence or absence of said mutation in the nucleic acid to a risk of developing hereditary lymphedema, wherein [the] presence of [a] said mutation [altering the encoded amino acid sequence or expression of at least one VEGFR-3 allele] in the nucleic acid correlates with an increased risk of developing hereditary lymphedema, and wherein absence of said mutation in the nucleic acid correlates with no increased risk of developing hereditary lymphedema.

2. [Amended] A method according to claim 1 or claim 37 wherein the assaying step comprises assaying for [determining the presence or absence of] a mutation altering a tyrosine kinase domain amino acid sequence of the protein encoded by the VEGFR-3 allele.

3. [Amended] A method according to claim 1 or claim 37 wherein the assaying step comprises [determining the presence or absence of] assaying for a missense mutation in a VEGFR-3 allele at a position corresponding to one of codons 857, 1041, 1044 and 1049 of the VEGFR-3-encoding sequence set forth in SEQ ID NO:1.

4. [Amended] A method according to claim 1 or claim 37 wherein the assaying step comprises [determining the presence or absence of] assaying for a missense mutation in a VEGFR-3 allele at a position corresponding to codon 1114 of the VEGFR-3-encoding sequence set forth in SEQ ID NO:1.

5. [Amended] A method according to claim 1 or claim 37 wherein said method comprises at least one procedure selected from the group consisting of:

(a) determining a nucleotide sequence of at least one codon of at least one VEGFR-3 allele of the human subject;

(b) performing a hybridization assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences;

(c) performing a polynucleotide migration assay to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences; and

(d) performing a restriction endonuclease digestion to determine whether nucleic acid from the human subject has a nucleotide sequence identical to or different from one or more reference sequences.

6. [Amended] A method according to claim 1 or claim 37 wherein said method comprises: performing a polymerase chain reaction (PCR) to amplify nucleic acid comprising VEGFR-3 coding sequence, and determining nucleotide sequence of the amplified nucleic acid.

7. [Amended] A method of screening for a VEGFR-3 hereditary lymphedema genotype in a human subject [patient], comprising the steps of:

(a) providing a biological sample comprising nucleic acid from said subject [patient], said nucleic acid including sequences corresponding to said subject's [patient's] VEGFR-3 alleles;

(b) [analyzing said nucleic acid for the presence of a mutation or mutations;

(c)] determining a VEGFR-3 genotype by analyzing said nucleic acid for the presence of a mutation altering the encoded amino acid sequence of at least one VEGFR-3 allele, wherein the presence of a mutation altering the encoded ~~a~~mino acid sequence of at least one VEGFR-3 allele of the human subject in a manner that reduces signaling of the VEGFR-3 polypeptide encoded by the allele identifies [from said analyzing step; and

(d) correlating the presence of a mutation in a VEGFR-3 allele with] a hereditary lymphedema genotype.

14. [Amended] An oligonucleotide useful as a probe for identifying polymorphisms in a human Flt4 receptor tyrosine kinase gene, the oligonucleotide comprising 6-50 nucleotides that have a sequence that is identical or exactly complementary to a portion of a [wild type] human VEGFR-3 gene sequence [or VEGFR-3 coding sequence] set forth in SEQ ID NO:1, except for one sequence difference selected from the group consisting of a nucleotide addition, a nucleotide deletion, or nucleotide substitution.

20. [Amended] An array of oligonucleotide probes immobilized on a solid support, wherein each probe occupies a separate known site in the array; and wherein the array includes at least one probe set comprising two to four probes, wherein one probe is exactly identical or exactly complementary to a [wild type] human VEGFR-3 coding sequence set forth in SEQ ID NO:1, and the other one to three members of the set are exactly identical to the first member, but for at least one different nucleotide, which different nucleotide is located in the same position in each of the one to three additional set members.

The following are NEW CLAIMS:

37. [New] A method of screening a human subject for an increased risk of developing hereditary lymphedema comprising assaying nucleic acid of a human subject for a mutation that alters the encoded amino acid sequence of at least one VEGFR-3 allele in a manner that correlates with the risk of developing hereditary lymphedema.

38. [New] The method of claim 37, wherein said mutation reduces signaling of the VEGFR-3 receptor.